

# Arterial-Venous Perfusion Without Anticoagulation to Reverse Accidental Hypothermia

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### Abstract

To investigate a means of rewarming individuals suffering from accidental hypothermia, five Yucatan swine were topically cooled to a core body temperature of 30°C. No heparin or any other means of anticoagulation was administered. An extracorporeal circuit consisting of polyvinylchloride tubing, a Bio-Pump and an external stainless steel heat exchanger was utilized and primed with an albuminized Ringers solution. An arterial-venous circuit was employed with oxygenation being provided by the animals' lungs. A series of aliquots of blood were taken and analyzed for coagulation prior to cannulation, after stabilization of blood flows, at a rectal rewarming temperature of 32°C and post-decannulation.

All subjects were effectively rewarmed to 34°C, with a mean perfusion time of 64.8 ± 8.5 minutes. Coagulation parameters showed very little alteration from the control values taken: PT (11.9 ± .5), PTT (16.0 ± .9), platelet count (456.0 ± 105.6) and normal fibrinogen levels. Necropsy examination revealed only minimal clot formation within either the extracorporeal circuit or the animals' vascular system.

Our results support the hypothesis that circulatory assistance and ultimately, extracorporeal circulation may be possible without anticoagulation.

### Introduction

Every winter, numerous individuals living in the colder regions, suffer life-threatening hypothermia because of exposure to the elements. An accepted method of treating these victims is with extracorporeal circulation (ECC) and rewarming,<sup>1-3</sup> necessitating the use of systemic anticoagulation. Should these individuals be the unfortunate recipients of traumatic insults, debilitating hemorrhage could be a result of

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the systemic anticoagulation.<sup>3,4</sup> Complications of such devastating hemorrhage are thrombocytopenia, cerebral hemorrhage, and death due to exsanguination.

The elimination of anticoagulation<sup>5</sup> in this modality of therapy would be a major contribution in reducing the morbidity and mortality associated with this procedure. An experimental animal protocol was designed and executed testing the following hypothesis: the moderately hypothermic subject could be successfully rewarmed using a modified cardiopulmonary bypass technique which excludes anticoagulation.

### Materials and Methods

Five Yucatan miniature swine,<sup>6</sup> with an approximate weight of 25 kilograms, were used. The animals were anesthetized using Acepromazine and Ketamine intramuscularly (IM); the appropriate level of surgical anesthesia was maintained with sodium pentobarbital intravenously (IV). One hundred percent oxygen was delivered through an endotracheal tube. When necessary, respirations were manually assisted with an Ambu-bag. A four-lead electrocardiogram (EKG) was placed on the anterior chest wall of the animal for continuous EKG monitoring throughout the procedure. An 18-gauge arterial pressure monitoring catheter was inserted into the animal's left femoral artery for arterial pressure recording and as an access for removal of blood for laboratory analysis. This consisted of activated clotting times (ACT), prothrombin time (PT), partial thromboplastin time (PTT), platelet count, fibrin split products, and fibrinogen level tests. The left femoral vein was cannulated as a vascular access for an intravenous infusion consisting of one gram of Lidocaine in 250cc of Dextrose 5% in water (D5W), which was allocated at a slow rate with the purpose of keeping arrhythmias at a minimum. Once the animal's right groin was dissected, the right femoral artery and vein were then cannulated for bypass. Subsequently, the animal was covered with ice and ice water in an effort to quickly reduce the core body temperature to between 28 and 30°C. This was measured by means of an indwelling rectal temperature probe. Both the

VARIABLE	MEAN	RANGE
Weight (kg)	25.6 ± 3.1	(23-31)
Bypass time (min)	64.8 ± 8.5	(55-76)
Pump flow (cc·m <sup>-1</sup> )	1280.0 ± 349.3	(800-1700)

All mean values are ± standard deviation.

*Table 1 - General Characteristics*

rectal and the heat exchanger water bath temperatures were monitored throughout the procedure. A second set of coagulation blood samples were drawn prior to the commencement of bypass.

The perfusion circuit consisted of appropriate lengths of 1/4" ID polyvinylchloride (PVC) tubing, a Bio-Pump Model BP80<sup>a</sup> and a stainless steel heat exchanger.<sup>b</sup> The circuit was primed with a solution that consisted of 12.5 grams of albumin in 500 cc of Ringer's solution, and was de-aired and recirculated for approximately 20 minutes prior to bypass. The perfusion circuit selected for this work was the drainage (outflow) line from the animal being conjoined to the femoral artery by means of a Bard 14-French arterial catheter.<sup>c</sup> The blood then flowed through the perfusion circuit and was perfused into the inferior vena cava through the right femoral vein catheter, a 14-French Bard catheter. This is an arterial-venous loop. Bypass was initiated once the "cold" lab work was completed. After establishing stable perfusion flows, rewarming commenced. We were careful not to exceed a temperature differential >10°C between the water in the heat exchanger and that of the blood entering the heat exchanger. Flows in the Bio-Pump in all subjects closely approximated the flow that one would expect for total body bypass in these subjects (> 60 cc/kg/min). The animals were maintained on bypass until the rectal temperature exceeded 34°C, at which time bypass was terminated. During

a Bio-Medicus, Inc., Model #BP80, Eden Prairie, MN

b Omnitherm Heat Exchanger, Model A-19-38

(SciMed Life Systems, Inc., Minneapolis, MN)

c Bard Cardiopulmonary Division, Billerica, MA

rewarming and again at the termination of perfusion, additional blood samples were collected and analyzed. All of the animals survived the procedure. The animals were electively sacrificed from 1 to 24 hours postoperatively, and necropsies were performed on all.

## Results

All animals survived the protocol; none displaying signs of excessive or unusual bleeding. A small amount of white clot was found in the inlet side of the Bio-Pump on the rim of the connector in Procedure #1. Procedure #2 had a minimal amount of white clot found in the inflow port to the heat exchanger, again located on the rim of the connection. None of the five animals showed any visible signs of clotting in the pulmonary artery or lungs.

Table 2 depicts the laboratory results of the blood samples that were performed. The activated clotting time (ACT) was by the method of Hattersley.<sup>7</sup> A normal range of 120 ± 10 sec for the pig, was found to be similar to that of a human's (120 ± 20 sec). Because a baseline ACT reading was drawn, each individual was able to act as its own control. This baseline value was drawn immediately after insertion of the arterial pressure monitoring catheter (mean 122.6 ± 6.8 sec) while the subject was normothermic. Additional ACT tests were drawn immediately prior to perfusion, and possessed a mean of 127 ± 5.9 sec. Once the rectal temperature reached 32°C during active rewarming, more tests were done with the results of a mean 121.8 ± 4.0 sec. Final ACT values were collected immediately once the rectal temperature had reached 34°C and perfusion had been terminated (mean 119.6 ± 7.2 sec). The widest variance found for a subject was in Procedure #5, in which an 11% change was found between the baseline ACT (110 sec) and the 32°C reading of 124 sec. Since both values were within normal limits, they were not significantly different. All other ACT values were essentially unchanged.

Table 2 also displays the results of the prothrombin time (PT) and the partial thromboplastin time (PTT) tests. It should be noted that no differences were detected in any of the tests performed while on bypass, nor was there any alteration from the control values (PT mean 11.4 ± .6 sec. and PTT mean of 15.8 ± 1.1 sec.). Table 3 (Fibrin Results) are laboratory results that quantitate an answer. These quantities that are being dealt with are directly dependent upon the size (mls) of the circulating volume. As this volume increases, these quantitated values will decrease in concentration, but not in totality, due to ECC techniques. Therefore, a mathematical adjustment must be employed. This adjustment, called indexing, is calculated by dividing the concentration number by the hematocrit value.<sup>8</sup> This reveals a value that is both reflective of the expanded volume, and the differential size of the animals. In Table 3, the quantitative fibrinogen levels are reported and then an indexed value follows. Except in Procedure #2, whereby cannulation problems required another cannulation site and re-introduction of perfusion, no differences were noted in the indexed values of quantitative fibrinogen. The fibrin degradation products remained unchanged throughout the procedure for all animals

VARIABLE	Baseline Control	During Bypass		After Bypass (34°C)	Significance* (ANOVA)
		Moderate Hypothermia	Mild hypothermia		
		(30°C)	(32°C)		
Platelet count (x 10 <sup>3</sup> )	620.2 ± 121.9	521.4 ± 66.4	441.6 ± 116.1	456.0 ± 105.6	NS
Platelet index	18.2 ± 4.6	16.4 ± 2.7	15.2 ± 4.6	14.9 ± 5.2	NS
ACT (sec)	122.6 ± 6.8	127.0 ± 5.9	121.8 ± 4.0	119.6 ± 7.2	NS
PT (sec)	11.4 ± 0.6	11.6 ± 0.6	12.0 ± 0.6	11.9 ± 0.5	NS
PTT (sec)	15.8 ± 1.1	16.4 ± 0.5	16.0 ± 0.9	16.0 ± 0.9	NS

ACT = activated clotting time; PT = prothrombin time; PTT = partial thromboplastin time.

All values are ± standard deviation; NS = not significant (p > 0.05).

Table 2 - Results of Coagulation Panel

(Table 3).

The platelet count values (Table 2) are another quantitative value and, therefore, must be altered to reflect the dilutional effects of perfusion. The absolute platelet count is divided by the hematocrit and carries the units of platelets/%. The absolute values are all within the normal range as are the corrected or indexed values, indicating no significant deviation from normal.

All animals were handled in compliance with the "Principals of Laboratory Animal Care," formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals," prepared by the National Institute of Health (NIH publication No. 86-23, revised 1985). Statistical analysis was conducted using SPSS PC Plus (version 2.0). Comparison between variables for each animal was assessed by analysis of variance (ANOVA) and t-test. Values are expressed as mean value plus or minus standard deviation. Statistical significance was considered for p values less than 0.05.

#### Discussion

The initial thesis was that induced hypothermia could offer some degree of coagulation protection for these laboratory animals. Five Yucatan miniature swine were subjected to this protocol. A prolongation of the ACT is a usual finding of clinical ECC.<sup>9</sup> The two causative agents that have been implicated in this prolongation are hemodilutional<sup>10</sup> and hypothermia.<sup>11</sup> It was anticipated that this, if actually occurring, might be enough of an alteration within the coagulation system to prevent clot formation. This prolongation in the ACT was not observed even though the desired end point of the protocol was

realized. A possible explanation for this could be that the ACT test warms the blood before clotting occurs, thus masking the desired effect by eliminating the hypothermic protection in the aliquot of blood being analyzed. A better test to use in this situation would probably be the bleeding time template,<sup>12</sup> which reveals the results regardless of temperature. Therefore, any effects that are directly attributable to hypothermia, would not be masked.

It is of interest that the animals were subjected to 65 minutes of ECC and heat exchange without the use of anticoagulants (Table 1), and survived without clot formation. Primarily, the protective mechanism was thought to be some sort of fibrinolysis, or fibrinolytic system involvement. But, since the index of fibrinogen level and the fibrin split product concentration did not change, this theory loses some credibility. It is presumed that the key to the lack of blood clotting in these circumstances is that all measured parameters appeared normal in an abnormal situation. Postoperatively, the animals appeared normal, with a normal coagulation panel. It is a well-known fact that hypothermia interferes with the metabolic processes of the body from the extensively detailed work done by many investigators. Hypothermia causes a dramatic decrease in the metabolism of the body. Gordon,<sup>13</sup> et al was able to demonstrate a 50% reduction in oxygen consumption when the body temperature declined from 37°C to 29°C. Blair<sup>14</sup> confirmed this with a 50-40% change in oxygen consumption at 30-28°C as compared to 37°C. This reduced utilization of oxygen transpires due to a reduced need of the tissues for oxygen. The reduced need for oxygen develops because hypothermia slows the

VARIABLE	Baseline Control	During Bypass		After Bypass (34°C)	Significance* (ANOVA)
		Moderate Hypothermia	Mild hypothermia		
		(30°C)	(32°C)		
Fibrinogen (mg/dl)	149.4 ± 40.1	127.8 ± 39.6	103.6 ± 42.3	106.2 ± 45.9	NS
Fibrinogen index	4.5 ± 1.8	4.1 ± 1.6	4.0 ± 1.8	4.2 ± 2.0	NS
Fibrin degradation products (mg/ml)	<8	<8	<8	<8	

All values are ± standard deviation; NS = not significant ( $p > 0.05$ )

### Table 3 - Fibrin Results

body's chemical reactions.<sup>15</sup> The pathways to coagulation are varied, but similar, in that the extrinsic, intrinsic and common pathways depend upon many chemical reactions in order to reach the end point.

Hypothermia also causes another aberration to occur. As the temperature of the blood decreases, the solubility of CO<sub>2</sub> increases.<sup>15</sup> This increasing CO<sub>2</sub> and a resultant increase in pH will create a much different environment within the body. Therefore, a pH of 7.4 at 37°C will become a pH of 7.52 at 29°C.

An enzyme is a protein that is synthesized in a living cell and has the function of catalyzing (speeding) a thermodynamic reaction so that the rate of reaction is compatible with the biochemical process essential for the maintenance of a cell.<sup>16</sup> It accomplishes this task by lowering the activation energy, and by increasing the number of molecules that are activated. In this way the equilibrium constant remains unaltered. Therefore, anything that could affect the equilibrium constant will also affect the rate of the enzymatically driven chemical reaction. Since chemical reactions are affected by temperature,<sup>15</sup> so is an enzyme-catalyzed reaction. pH changes will profoundly affect the catalytic nature of these enzyme reactions. This pH fluctuation may cause denaturation and hence inactivation of the enzyme proteins. It is conceivable that the enzymatic reactions necessary for coagulation have been chemically (hemodynamically) slowed due to the altered environment induced by the procedure.

Tanaka, et al, discovered that the coagulation system is

activated during bypass even in the presence of heparin, and that the origin is that of the extrinsic coagulation cascade.<sup>17</sup> The tissue plasminogen activator antigen (t-PA) released from the injured vascular endothelium is the fibrinolytic response to the extrinsic activation and is the marker used in determining which system is involved. t-PA is an enzyme that is released from damaged vascular endothelium. Therefore, it is conceivable that the hypothermic changes slowed the rates of reaction of the extrinsic pathway to such a degree that clotting did not occur.

By pre-coating the ECC surface with albumin, a major prime constituent, it was thought that any damaging effects of a foreign surface activated clot (intrinsic pathway) could be ameliorated. This pseudoendothelium can develop due to the opposing electrical attractions between the various molecules and may result in an inner layer composed of blood proteins.<sup>13</sup> Platelets have been rendered essentially inactive due to hypothermia reversibly repressing the production of thromboxane A<sub>2</sub>.<sup>19</sup> Additionally, it is suspected that hypothermia directly or indirectly may affect the reactivity of factor XII to the extent of not causing platelet aggregation even though other predisposing factors are present.<sup>20</sup>

The slowing of the rate of reaction by hypothermia, alteration in the blood proteins due to a change in pH and the cold inactivated platelets all combine to protect against clot formation in the hypothermic state. But, what about in the rewarming phase and normothermic state?

Zilla,<sup>8</sup> et al, concluded that platelet stimulation occurs primarily at the onset of perfusion and probably returns to

normal during bypass. Since the stimulated platelets were exposed to the hypothermic state pre-pump, this platelet stimulation may have been tempered to a large extent. Blood flow rate (Qb) plays an extremely important role in the scenario. In this protocol, during the rewarming phase, blood flow rates, at a minimum, often exceeded 80 cc/min/kg. Toomasian<sup>21</sup> and Dixon<sup>22</sup> both argue strongly for high blood flow rates. In one procedure, clots formed when the blood flow fell below 2 L/min/m<sup>2</sup>. Whittlesey<sup>23</sup> agrees with this and argues that anticoagulation with heparin may not be necessary for ECC. A contention that needs further collaborative efforts before substantiation is provided. In this protocol, judicious attention was directed toward a circuit as diminutive in size as practicable. This allowed for a rapid transit time with absolutely minimal areas of stagnation. This in combination with high blood flows helped to avoid the deleterious events that allow blood to clot. The very reason that this arterial-venous (AV) circuit was selected was in keeping with the premise of maximal blood flow. The blood flow into the Bio-Pump was enhanced due to the arterialized pressure thus greatly increasing pump pre-load. By re-infusing into the venous site, a greatly reduced after-load was recognized thus allowing for a higher blood flow, with minimal resistance.

#### Conclusion

An attempt to develop an extracorporeal system capable of rewarming the individual without the use of any form of anticoagulation was investigated. By utilizing the Bio-Medicus BP80, a heat exchanger and PVC tubing, we were able to rewarm five experimental animal models without obvious sequelae. As long as blood flow was maintained above a minimum level in the extracorporeal circuit, clotting did not appear to be a significant problem. However, once extracorporeal flow ceases, decannulation must be accomplished immediately. In an experimental series utilizing dogs and then sheep, Magovern<sup>24</sup> had similar results to those discussed here. In an attempt to develop a heparinless ventricular assist system, they apparently did not have to deal with a heat exchanger nor temperature gradients in their protocol. Building upon their previous work, they now utilize a form of this technique when using extracorporeal techniques to support the failing heart.

It became apparent that the coagulation system of these animals was not altered, yet did not clot while on bypass. Protection from clotting offered by hypothermia, induced by pH changes, or avoided by high flows, have been scrutinized. Controlled fibrinolysis a condition wherein the activation stimulus to the clotting system is so minute as to be overshadowed by the fibrinolytic system, has been suggested as the protective mechanism. Further elaborative work is being conducted in order to elucidate the appropriate biological mechanisms involved and the alterations they have endured.

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